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Eutypella phaeospora, a new species on *Chenopodiaceae*

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Abstract — The authors describe a new species of *Eutypella* (*Diatrypaceae*, *Xylariales*) based on several collections on *Suaeda vera* in France and one on *Salsola vermiculata* in Spain. The *Libertella*-like asexual state was obtained in culture.

Key words — *Ascomycota*, taxonomy, salt marsh, disjunct distribution, semi-arid land

Introduction

An ascomycete referable to *Eutypella* (Nitschke) Sacc. was repeatedly collected on *Suaeda vera* J.F. Gmel. (*Chenopodiaceae*), a woody shrub occurring in salt marshes in Ile de Ré (France), and once on *Salsola vermiculata* L. (*Chenopodiaceae*) in a semi-arid environment in northern Spain. Salt marshes are known to be peculiar ecosystems in which halophytes associate with both terrestrial and marine mycota (Barata 2002). Previous investigations have been carried out mainly on monocots such as *Juncus roemerianus* (Kohlmeyer et al. 1995), *Spartina* sp. (Gessner & Kohlmeyer 1976), *S. alterniflora* (Gessner 1977), and *S. maritima* (Barata 2002), but little attention has been paid to woody shrubs.

Based on extensive collecting of ascomycetes in Ile de Ré, this *Eutypella* appears to be somewhat common in salt marshes but was never encountered on the island out of this biotope. However, its occurrence on *S. vermiculata* in a very different environment in Spain suggests a stronger relationship to *Chenopodiaceae* than to a specific ecological niche as first assumed.

As it is also remarkable in possessing ellipsoid brown ascospores, we decided to describe it as new. A typically diatrypaceous anamorph obtained in culture confirmed its placement in *Diatrypaceae* and a living culture was deposited in CBS, allowing further phylogenetic investigations in the future.

Materials & methods

Macro and microscopic observations were carried out following Rappaz's (1987) methodology, especially by testing the reaction of subapical ring to Melzer's reagent after pre-treatment in 3% KOH and by making measurements of asci and ascospores in heated lactic cotton blue. Micrographs of asci were taken in Lugol's solution (IKI), those of conidiogenous structures and conidia were taken in dilute Waterman blue ink and water. Cultures were made from single ascospores which were isolated on malt extract agar (Difco™ Malt Extract Agar) according to Rossman's (1999) method. The material collected, along with duplicates of holotype, is kept in the the authors' private herbaria (JF and CLL numbers). The holotype is deposited in LIP herbarium (Lille) and cultures at CBS.

Taxonomy

Eutypella phaeospora J. Fourn. & Lechat, **sp. nov.** Plate 1

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Ab alteribus speciebus generis Eutypella *differt combinatione crescentiae in* Chenopodiaceae*, ascosporarum ellipsoidearum et brunneorum et apiculi annuli leviter amyloidei*.

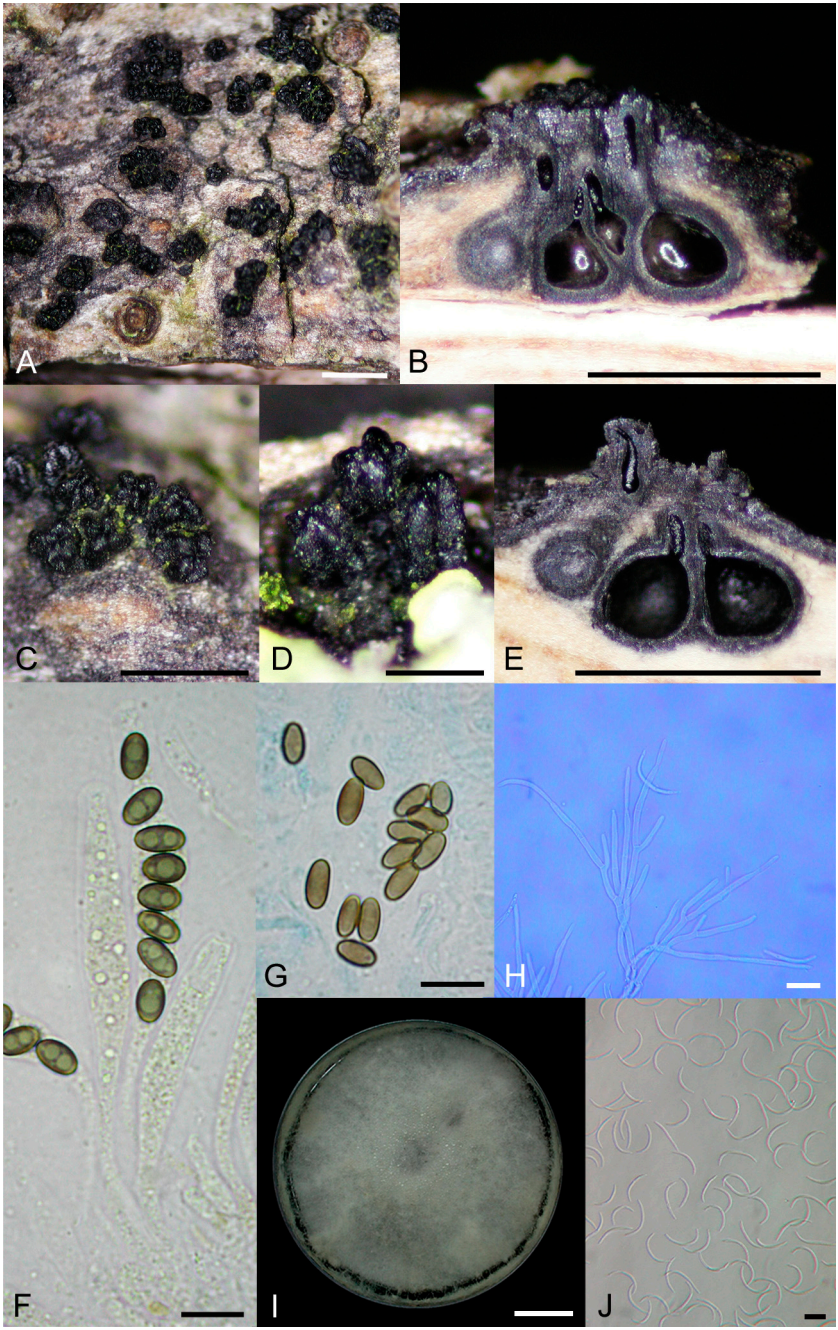
Type: France, Charente Maritime: Ile de Ré, Loix, Le Feneau, edge of a salt marsh, on dead or dying stems of *Suaeda vera*, 16 Apr. 2008, leg. MH, CLL & JF (**Holotype**, LIP JF08078; ex-type culture, CBS129148).

Etymology: The epithet is derived from the unusual brown colour of ascospores.

Stromata corticolous or lignicolous, pustulate, pustules 0.8–1.8 mm diam, comprising 2–8 perithecia, irregularly scattered, separate to sometimes confluent; in bark, stromata irregularly rounded, hardly raising the periderm, under a conspicuous dorsal black line which is absent when the periderm is tightly adherent, cortical tissue hardly altered, without differentiated entostroma; in wood, stromata fully immersed to slightly raising wood surface, elongated in the grain of wood, substrate not altered but surface more or less blackened. Ostioles emerging collectively as small bunches 0.5–1 mm diam, shiny black, rectangular with cruciform apices and deeply sulcate sides, (80–) 150–840 µm high, 210–290 µm diam. Perithecia subglobose, 380–420 µm diam, in contact, with short to long convergent necks. Asci unitunicate, thin-walled, long-pedicellate, the spore-bearing parts cylindrical to fusiform

Plate 1. *Eutypella phaeospora* (holotype). A: Surface view of host bark showing bunches of ostioles erumpent through the periderm. B: Cross section through a stroma in bark showing convergent ostiolar necks. C–D: close-ups showing the stout cruciform ostioles. E: Cross section through a stroma in wood showing convergent ostiolar necks. F: Mature and immature asci mounted in Lugol's solution showing the very faint reaction of apical ring. G: Ascospores mounted in lactic cotton blue. H: Conidiophores, conidiogenous cells and conidia of the *Libertella*-like anamorph mounted in dilute Waterman blue ink. I: Culture in Petri dish. J: Conidia in water.

Scale bars: A = 5 mm; B–C,E = 1 mm; D = 0.5 mm; F–H,J = 10 µm; I = 1 cm.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with a truncate apex, 35–40 µm long × 7.5–8.5 µm broad with a minute, very inconspicuous subapical ring bluing in Lugol's solution or in Melzer's reagent after pre-treatment with 3% KOH, the stipes 30–35 µm long, filiform, very fragile. Paraphyses copious, persistent, hyphae-like, hyaline, septate, 4–5 µm at base, tapering above asci. Ascospores 6–7.5 × 3.4–4 µm, oblong to ellipsoid-equilateral with broadly rounded ends, brown, one-celled, smooth, obliquely uniseriate to irregularly biseriate in the ascus.

Additional specimens examined: **FRANCE**, **Charente Maritime**: Ars en Ré, edge of a salt marsh, on dead or dying stems of *Suaeda vera,* 07 May 2007, leg. C. Lechat, CLL7129. **SPAIN**, **Navarra**: Arguedas, 80 km south of Pamplona, on roadside in the semi-desert of Bardenas Reales, 310 alt., on a dead corticated stem of *Salsola vermiculata*, 26 Sep. 2010, leg. Jean-Paul Priou, CLL10033.

Anamorph in culture: After two months on MEA, colony wrinkled, 2.5–3 cm diam., 0.3–0.5 cm high, producing a *Libertella*-like anamorph, white at first, then covered by greyish-white hyphal elements, white at margin, diffusing a brown to blackish coloration in the medium; reverse side greyish-yellow in the center, blackish at margin. Conidiophores branched, conidiogenous cells cylindrical, producing holoblastic conidia 16–23(–26) × 1.1–1.3 µm, hyaline, white en masse, cylindrical, tapered at ends, irregularly curved, e.g. curved on upper third of length or regularly and strongly curved, produced in whitish clusters on the median part of the colony.

Discussion

This ascomycete meets all the key features of the family *Diatrypaceae* Nitschke in macromorphology, ascal morphology, and *Libertella*-like anamorph. It only deviates in possessing ascospores that are ellipsoid and brown instead of allantoid and yellowish as encountered in typical taxa. However, several species in *Anthostoma* Nitschke, *Cryptosphaeria* Ces. & De Not., *Diatrype* Fr. and *Eutypella* are known to have brown ascospores (Rappaz 1987, 1992), and several of them —*A. decipiens* (DC.) Nitschke, *C. subcutanea* (Wahlenb.) Rappaz, *D. whitmanensis* J. D. Rogers & Glawe, *E. grandis* (Nitschke) Sacc., *E. corynostomoides* (Rehm) Rappaz— produce such ellipsoid ascospores along with broadly allantoid ones (Rappaz 1987).

The new taxon appears best accommodated in *Eutypella* as defined by Rappaz (1987) based on its pustulate stromata with stout, converging ostiolar necks. Although the recent ITS sequence analysis of *Diatrypaceae* by Acero et al. (2004) showed that *Eutypella* as currently conceived is most likely polyphyletic, those authors did not propose any formal taxonomic changes. We therefore refer the present fungus to *Eutypella* and await further molecular studies that might shed some light on its affinities within *Diatrypaceae*. A case was made to reconsider its placement following Carmarán et al. (2006), who reinstated *Peroneutypa* Berl. for taxa differing from *Eutypella* in ascal morphology.

Although we did not use fluorescence microscopy to study the morphology of asci in *E. phaeospora*, the ascal shape and subapical ring clearly point towards type I as defined by the authors and therefore suggest a closer affinity with *Eutypella* as currently accepted (Rappaz 1987).

Eutypella phaeospora can be readily distinguished from other *Eutypella* species through its consistently ellipsoid brown ascospores and occurrence on members of *Chenopodiaceae*. The usually uniseriate ascospore arrangement in the ascus correlated with the cylindrical shape of its spore-bearing part is likewise a distinctive feature contrasting with the spindle-shaped ascus most often encountered in *Diatrypaceae*. The fungus seems to be a common saprobe occurring on *Suaeda vera* in France and on *Salsola vermiculata* in Spain.

Eutypella kochiana Rehm and *E. alsophila* (Durieu & Mont.) Berl. (which occur on *Chenopodiaceae* in marine environments) are worth notice here, but their asci have a clearly amyloid subapical ring and their ascospores markedly differ in being yellowish and allantoid. *Eutypella kochiana* var. *salsolae* Urries, collected on *Salsola vermiculata* in Spain, resembles *E. phaeospora* in slightly larger ascospores (4.8–8 × 1.8–2.2 µm —vs. 4.8–6 × 1.5–1.8 µm in *E. kochiana*) that are sometimes oblong to broadly ellipsoid (Rappaz 1987). However that variety still differs in having asci with a clearly amyloid subapical ring and narrower, yellowish ascospores. The above collection of *E. kochiana* var. *salsolae* does suggest that at least two different *Eutypella* species do occur on *Salsola vermiculata*, whereas *E. phaeospora* is the only *Eutypella* species known thus far to occur on *Suaeda vera*.

Based on many occurrences of *E. phaeospora* on *S. vera* in salt marshes in Ile de Ré and absence inland, it was first assumed that the new species was restricted to coastal environments. However, the material collected in Spain from a very different environment —a semi-arid area far inland— points towards a disjunct distribution not correlated with salt marshes. The fact that *Salsola vermiculata* is an invasive shrub that can thrive in both coastal wetlands and inland semi-arid areas may explain this apparent discrepancy. Therefore, it could be assumed that *Eutypella phaeospora* exhibits a strong preference for *Chenopodiaceae* species regardless of ecological niche. Occurrence of *E. phaeospora* on *S. vermiculata* in salt marshes is predictable but should be confirmed by further sampling in coastal areas. Whether *E. phaeospora* occurs on other members of *Chenopodiaceae* or possibly on other shrubs is likewise still unknown, and it is hoped the morphological characterization given above will help elucidate its apparently complex ecology.

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